# SMRT<sup>®</sup> Link release notes (v11.1)



**Note**: SMRT Link v11.1 is for use with Sequel<sup>®</sup> II and Sequel IIe systems **only**. Customers running Sequel systems, or those with mixed fleets running Sequel and Sequel II/IIe systems, should continue using SMRT Link v10.2.

### SMRT Link server installation

SMRT Link server software is supported on:

- English-language CentOS 7.x, supported until end-of-life 6/30/2024.
- English-language Rocky Linux 8.x supported until end-of-life 5/31/2029.
- Ubuntu 18.04 and 20.04 64-bit Linux® distributions.
- These supported versions **also** apply to SMRT Link compute nodes.
- Note: PacBio® advises against new installations of CentOS for use with SMRT Link.
- SMRT Link is not guaranteed to work on Linux versions that are no longer supported by the operating systems' vendors.
- SMRT Link server software cannot be installed on systems running other versions of UNIX, macOS<sup>®</sup> or Windows<sup>®</sup>.
- Several SMRT Link v11.1 features are computationally-intensive and require adherence to the computational and storage hardware requirements listed in the document SMRT Link software installation guide (v11.1).

To install **only** command-line SMRT<sup>®</sup> Tools, use the --smrttools-only option with the installation command, whether for a new installation or an upgrade. See **SMRT Link software installation guide (v11.1)** for complete instructions.

## Supported chemistry

• SMRT Link v11.1 supports all chemistry versions for Sequel II and Sequel IIe systems.

#### SMRT Link database note

• SMRT Link v11.1 no longer includes weekly automatic database backups. A database backup is still automatically performed once, during installation or upgrade. Failure to back up the SMRT Link database on a regular schedule risks losing all records in SMRT Link (including users, Data Sets, analyses, barcodes, and references) if a file system or reconfiguration error occurs. The underlying sequencing or analysis files, such as BAM files, are **not** affected. We **strongly** recommend asking your local Linux system administrator to schedule regular weekly backups of the SMRT Link database using standard Linux utilities. For additional details, please contact PacBio Technical Support.

# **New Features**

## SMRT<sup>®</sup> Analysis support for new MAS-Seq for 10x Single Cell 3' kit

- New **Single-cell Iso-Seq**<sup>®</sup> **Analysis** application enables easy analysis and functional characterization of full-length transcript isoforms with additional single-cell information, including single-cell barcodes and unique molecular identifiers (UMIs).
- New **Read Segmentation** data utility splits arrayed HiFi reads at concatenation adapter positions, generating segmented reads (S-reads) which are the comprising fragments.

 New Read Segmentation and Single-cell Iso-Seq Analysis application combines the functionality of the Read Segmentation data utility and the Single-cell Iso-Seq Analysis application. This application is for use with concatenation-based library preparations such as the MAS-Seq libraries.

# SMRT Tools support for new MAS-Seq for 10x Single Cell 3' kit

- New skera deconcatenation tool splits arrayed HiFi reads at adapter positions, generating segmented reads (S-reads) which are the individual fragments.
- New pigeon PacBio transcript toolkit contains tools to classify and filter full-length transcript isoforms into categories against a reference annotation.
- Updated isoseq3: Added support for the new single-cell Iso-Seq workflow.

## Run Design support for new MAS-Seq for 10x Single Cell 3' kit

New MAS-Seq single-cell Run Design application choice to support the new MAS-Seq for 10x Single Cell 3' kit.

# Sample Setup support for new MAS-Seq for 10x Single Cell 3' kit

• New **MAS-Seq single-cell** Sample Setup application choice to support the new MAS-Seq for 10x Single Cell 3' kit.

#### **SMRT Analysis**

- New **Summarize Jobs** functionality generates summary information on multiple selected **Microbial Genome Analysis** jobs. (In this release, only the **Microbial Genome Analysis** workflow is supported. Future releases will include support for summarizing additional workflows.)
- The Minor Variants Analysis pipeline was deprecated in this release.

# **Known Issues**

- Reference download fails if the curl command is not installed on the server operating system.
- Large runs POST updates are sometimes rejected by the API gateway.
- The AWS Datasync Lambda function python runtime should be upgraded to v3.9 as the current v3.8 is no longer supported.
- Single-cell Iso-Seq: The Read Statistics reports FL-tagged reads with a misleading title.
- **Single-cell Iso-Seq**: When uploading a custom 10x barcode in the **Advanced Parameters** dialog, the workflow should block users from doing anything until the upload is completed.
- **Single-cell Iso-Seq**: When an uncompressed 10X barcodes file is uploaded using the **Advanced Parameters** dialog, the upload is rejected, but the GUI still shows the upload as being successful.
- **Single-cell Iso-Seq**: The input of isoseq3 bcstats should be the output of isoseq3 correct.
- **Sample Setup**: Creating a new high-throughput calculation without specifying an application displays an erroneous error message.
- Sample Setup: Sometimes cell and volume validation messages are inconsistent.
- Run Design: Run Designs that specify both Include Low Quality Reads and Include Kinetics Information generate a server error.
- **Run Design**: When creating a new Run Design and selecting an application, sometimes incorrect values are entered into the Run Design fields.

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- Run Design: When saving a new Run Design, an error message is sometimes displayed.
- Run Design: The Run design Auto Analysis GUI doesn't display the expected Data Set members.
- **Run Design**: It is possible to import a Run Design .CSV file that specifies too many reaction plates. The Run Design will load on an instrument, but then fail the inventory scan.
- Windows line endings from demultiplexing jobs are inserted into the @RG SM field of BAM file headers. Such line endings can cause unexpected issues with downstream tools that parse BAM file headers.
- The skera tool only processes the first BAM file in a multi-cell Data Set.
- Multi-job submissions using the GUI are run on the head node instead of using distributed processing.
- When clicking **Start and Create Next** for multiple-Data Set custom parameters, the demultiplexed output Data Set name is not updated to reflect the newly selected Data Set.
- When creating an Auto Analysis, selecting a Run, then clicking **Back** enables workflow choice.

# **Fixed Issues**

- Fixed an issue where the <code>\$SMRT\_ROOT/admin/bin/keytool</code> symlink pointed to a non-existing file.
- Fixed an issue that caused the Trim Ultra-Low Adapters data utility to fail.
- Fixed an issue that caused a Microbial Genome Analysis job to fail.
- Fixed an issue where a Microbial Assembly job with two combined Data Sets produced a single, small contig.
- Fixed an issue where the imported .CSV setting was not retained when the run was modified in the GUI.
- Fixed an issue where the SMRT Analysis Iso-Seq report primer data table displayed the same numbers for different multiplexed samples.
- Fixed an issue where using **Data > File Downloads > Edit Output File Name Prefix** to add a Data Set name prefix did not work.

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